Hey1, DLL4 x Hes1, and Notch1 x Hes1; ($p < 0.01$)] that may confer CAD risk.

Conclusions: The Notch pathway is activated in atherosclerotic plaques and results in endothelial inflammation and senescence. Notch signaling may be linked to CAD risk. These findings implicate, for the first time, a potential involvement of Notch signaling in Atherosclerosis.

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PS202.

Elevated Peri-Operative C-Reactive Protein Impedes Late Vein Graft Remodeling

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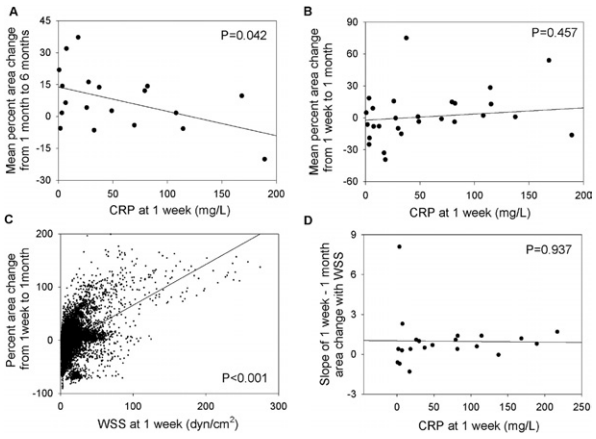
Objectives: Enhanced systemic inflammation, as measured by C-reactive protein (CRP), has been associated with reduced early outward remodeling and long-term vein graft (VG) failure. This study seeks to define the long-term changes in VG remodeling that are induced by this pro-inflammatory phenotype.

Methods: A prospective study was performed on 31 patients undergoing autogenous VG placement. CT scans, with computational analysis of wall shear stress (WSS), were performed at 1wk, 1m, and 6m to evaluate lumen remodeling. hsCRP was obtained pre-op and 1wk post-op.

Results: Late changes in VG lumen diameter (between 1m to 6m) were negatively correlated with CRP levels obtained at 1wk post-op (Fig A, P=0.04), while early changes in VG diameter (1wk and 1m) were independent of 1wk CRP (Fig B, P=NS). Adaptation of the lumen was positively correlated with WSS (Fig C, P<0.001) and was not influenced by the 1wk CRP level (Fig D, P=NS). Neither early or late geometric VG changes nor the adaptive response to WSS were correlated with pre-op CRP levels. Six (of 31) VG were revised or occluded within 1 year, and this was not dependent on either pre-op or 1wk CRP.



Conclusions: Late VG remodeling was significantly reduced by an enhanced peri-operative inflammatory response, while early VG adaptation was independent of this response. In contrast to published reports, we found no correlation between the baseline inflammatory state of the patient and WSS-dependent VG adaptation. The current studies suggest long-term structural modulation of the VG by the inflammatory system as the dominant determinant for these events.

Author Disclosures: S. Berceli: Nothing to disclose; **Y. He**: Nothing to disclose; **M. Hong**: Nothing to disclose; **P. Nelson**: Nothing to disclose; **R. Tran-Son-Tay**: Nothing to disclose.

PS204.

The Toll-like Receptor 2 Ligand HMGB-1 Contributes to Skeletal Muscle Damage in Critical Limb Ischemia

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Objectives: Inflammation and cell damage contribute to the pathophysiology of critical limb ischemia (CLI). Toll-like receptors (TLRs) play an important role in inflammation and tissue damage probably in response to the release of endogenous ligands. We hypothesize that the expression of TLRs and their endogenous ligands is upregulated in ischaemic skeletal muscle with consequent activation of their signaling pathway, which could lead to an increase in inflammatory cytokine release contributing to muscle damage.

Methods: TLR expression was studied in ischaemic and control human muscle biopsies and in C2C12 myotubes cultured in ischaemic conditions using RT-PCR and Western blot. Western blot was used to measure the expression of the endogenous ligand, high mobility group box protein-1 (HMGB-1). Functional effects of TLR2 antagonism on ischaemia-induced IL-6 release and cell death were studied by incubating myotubes with neutralizing TLR2 antibody. IL-6 release was assayed by ELISA. Apoptosis was assessed using cleaved caspase-3 and bax/bcl-2 ratio measurements.

Results: TLR2 mRNA and protein expression was significantly upregulated in ischaemic muscle and in C2C12 myotubes cultured in ischaemic conditions ($p<0.05$). Raised levels of HMGB1 were demonstrated in ischaemic human muscle biopsies and in ischaemic C2C12 myotubes.TLR2 antagonism reduced ischaemia-induced IL-6 production and apoptosis in culture.

Conclusions: Upregulation of TLR2 and HMGB-1 expression occurs in ischaemic muscle. Activation of TLR2 signaling leads to IL-6 release which may contribute to inflammation and muscle damage. HMGB-1 inhibition may be a potential target in reducing skeletal muscle damage in CLI.

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PS206.

"Back-Table" Manipulation of Human Saphenous Vein Significantly Impairs Endothelial and Smooth Muscle Function

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Objectives: Human saphenous vein (HSV) is the most widely used arterial bypass conduit despite a high rate of intimal hyperplasia (IH). IH is thought to evolve as a response to vascular injury. We investigated whether injury from back-table surgical preparation impairs HSV endothelial and smooth muscle function.

Methods: HSV segments were collected from patients undergoing arterial bypass. HSV was collected after surgical removal but before manipulation (unmanipulated, UM). Additional HSV was collected after back-table manipulation, which included hand-held syringe distention and marking for orientation (after manipulation, AM). Paired UM and AM segments were obtained from 15 patients. HSV rings were suspended in a muscle bath. Force measurements were obtained after administration of phenylephrine (PE), sodium nitroprusside (SNP), and carbachol (CCH).

Results: PE induced mean force of 0.07±0.04 N/m^2 in UM-HSV, versus 0.03±0.04 in AM-HSV (p=0.001). SNP induced smooth muscle dependent relaxation of 53.9±23.9% in UM-HSV, versus 21.4±16.6 in AM-HSV (p<0.0001, Figure). CCH induced endothelial dependent relaxation of 19.9±12.5% in UM-HSV, versus −0.7±6.3 in AM-HSV (p=0.001, Figure).

Conclusions: Back-table preparation causes injury which markedly decreases HSV endothelial and smooth muscle function. This argues for less injurious means of distending and marking HSV grafts.

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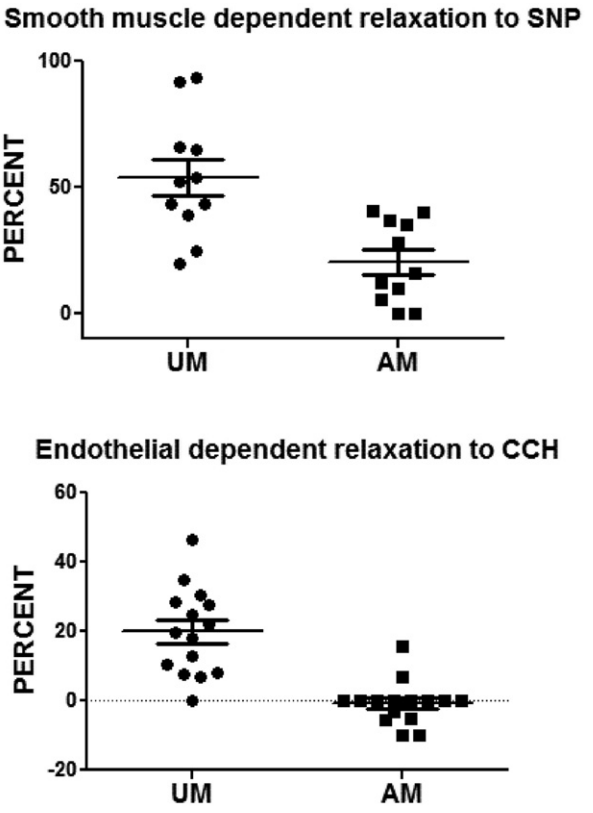

Figure. Percent relaxation of UM versus AM HSV in response to SNP (p<0.0001), and CCH (p=0.001).

M. J. Osgood: Nothing to disclose; **K. W. Sexton**: Nothing to disclose.

PS208.

Development of a Non-Viral, Non-Toxic Method for Gene Therapy in Vascular Smooth Muscle Cells

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Objectives: Gene therapy shows promise in the treatment of vascular disease, but a formidable challenge has been delivery of genetic material in a safe and non-toxic way. Viral transfection comes with significant clinical implications in terms of safety, and there are still no FDA-approved gene therapy products. This has led to recent interest in developing alternatives to viral transfection that are low risk, predictable, and non-toxic. Biodegradable polymers have shown promise as one of these alternatives, but to date have been exclusively tested in human stem cells. Differentiated cell types would be prime targets for therapeutic gene modulation in the prevention of various disease processes. We aim to establish polymeric transfection as a method of gene therapy in cells of vascular origin.